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THE DEVELOPMENT OF THE OVOTESTIS AND COPULATORY ORGANS IN A POPULATION OF PROTANDRIC SHRIMP, *PANDALUS PLATYCEROS* BRANDT FROM LOPEZ SOUND, WASHINGTON [1]

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The large commercially important shrimps of the decapod family Pandalidae demonstrate high incidences of protandric hermaphroditism (Berkeley, 1929, 1930). Almost all of the individuals pass through a functional male stage of one to three years before undergoing transformation to a functional female stage. During the male stage the gonad is an ovotestis, but only the testicular elements are functional. Whereas, in the female stage the gonad is a true ovary, the testes having degenerated during sexual transformation.

The incidence of protandry among the eleven different species of *Pandalus* and *Pandalopsis* from the North Pacific appears to be variable in those populations that have been studied. Some species are dominantly protandric such as *Pandalopsis dispar, Pandalus platyceros,* and likely *P. montagui tridens* and *P. stenolepis* (Butler, 1964). Primary females or those females that have not passed through an initial male stage have been reported in populations of *Pandalus jordani, P. hypsinotus, P. danae* and *P. goniurus* (Butler, 1964); the circumpolar *Pandalus borealis* (Allen, 1959; Carlisle, 1959a, 1959b, 1959c; Butler, 1964); *Pandalus montagui* from North Atlantic and North Sea populations (Leloup, 1936, Mistakidis, 1957; Allen, 1963). Aoto (1952) has recorded that the Japanese pandalid, *Pandalus kessleri* appears to be dominantly protandric.

The known gonochoristic species generally are found in North Atlantic populations. These include *Pandalus bonnieri* (Pike, 1952) and *Pandalus propinquus* (Jägersten, 1936). There is also evidence that certain populations of *Dichelopandalus leptocerus* are gonochoristic (Scattergood, 1952).

Although Butler (1964) and Berkeley (1930) have made intensive studies on various populations of different species of pandalids off the coast of British Columbia, there remains questions that need to be answered concerning the relations between the age classes of shrimp and the stage of gonadal differentiation. Also, further information is needed on the relationship between age and size to the phenomenon of sexual transformation.

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Materials and Methods

Pandalus platyceros Brandt is the largest of the Pacific pandalids. The females at times exceed 20 cm in length from the base of the eyestalk to the tip of the telson. This shrimp is fished heavily by commercial shrimpers throughout its range from Unalaska south to San Diego. However, the important fishing is found in the inland waters off the coast of Alaska, British Columbia, Washington and Oregon. The coloration of the shrimp is quite variable ranging from a blue-green generally found in small immature individuals to a brilliant orange. Regionally, *P. platyceros* is commonly called "The Spot" owing to a pair of white spots on the first and fifth abdominal pleura. The adults are generally found in water to a depth of 266 fathoms, but the larvae and immature forms are found in shallow inlets (Berkeley, 1930; Butler, 1964). Fishing for these shrimp is done either with the aid of trawls or shrimp pots. Butler (1964) states that the female stage is not reached until the fourth or fifth summer.

The shrimp that were utilized in this study were taken from a population that can be found in Lopez Sound, Washington, which is one of the many shallows inlets of the San Juan Archipelago. There is a depression or hole in the south central region of the sound that ranges in depth from 15 to 27 fathoms. It is in this region that abundant numbers of shrimp were found. In addition to *P. platyceros*, a large number of other species of shrimp were taken. They include, in order of relative abundance, *P. danae*, *P. goniurus*, *P. hypsinotus* and *P. stenolepis*. During the winter months, immature specimens of *Pandalopsis dispar* were also present. Although the exact number of different species was not determined, many species of hippolytid shrimp of the genera *Eualus*, *Spirontocaris*, and *Lebbeus* were present in this depression. In addition, a few species of *Crangon* were found.

Collection of the shrimp which generally was made on a bi-monthly basis over the period of three years (1965–1968) with the aid of a ten foot shrimp trawl.

In the laboratory, the shrimp were maintained in twenty to thirty gallon plexiglas aquaria that were supplied with running sea water. For histological studies, generally the shrimp were sacrificed within a few days of collecting. About 1,284 animals were utilized in this study.

The gonads were fixed both for wax and epoxy sections. Several different fixatives were employed for wax sections. The order of their effectiveness to fix the tissue were: Helly's, Heidenhain's "Susa," Stieve's, Zenker's and Bouin's. Helly's fixative gave the best cytoplasmic fixation whereas Heidehain's "Susa" and Stieve's fixatives were best for nuclear fixation. After initial dehydration in increasing concentrations of ethanol and tertbutyl alcohol, the tissue was enbedded in Paraplast. Sections six to seven microns were cut and mounted on albuminized slides. The stains used most frequently were the following: Gomori's Chromium Hematoxylin and Phloxine (Gomori, 1941), a modified Azan technique (Hubschmann, 1962), the Periodic Acid Schiff technique of Purves and Grieshbach (1951), and the technique employed by Halmi (1952) which was used and without the paraldehydefuchsin stain. A glutaraldehyde fixation was used for epoxy sections. Primary fixation in glutaraldehyde and postfixation in osmium is outlined in Hoffman (1969). One-half to one micron sections were strained for light microscopy using Richardson's stain (Richardson, Jarrett and Finke, 1960).

The following measurements and indices were used to determine the age and sex of the shrimp:

(1) Carapace length was used as a measurement of the size and age class. The measurement is centimeters was taken along the left side of the carapace from the base of the eyestalk to the posterior edge of the carpace (Butler, 1964). Total length proved to be too variable an index of the age of the shrimp since the lengths of the rostrum and abdomen varied in some shrimp with identical carapace lengths.

(2) The degree of development of the gonad and the sexuality of the shrimp were determined by means of histological sections. Transverse and frontal paraffin sections were made to determine general morphology and thick plastic sections of glutaraldehyde and osmium fixed tissue were made to determine the cytological aspects of the gonads.

(3) The sexuality of the shrimp was also determined by whole mounts of the copulatory organs of the first two pair of pleopods. Correlations could then be made between the secondary sex characteristics and the primary sex characters of the gonad.

Results

The development of the copulatory organs

As in all natantian decapods, the male copulatory organs are located on the first two pair of pleopods. In a functional male the copulatory organ of the first pleopods consists of a swollen bulb at the distal end of the endopodite. This organ has its dorsal and lateral surface covered with hooks or cincinnuli measuring about 50 microns in length. These hooks appear to aid the shrimp in the transfer of the spermatophores to the female. The copulatory organs of the second pair of pleopods are located at the distal end of the protopodite, lateral to the endopodite. Each organ consists of two finger-like rami; the more medial is called the appendix interna and the more lateral is called the appendix masculina. The appendix interna is devoid of spines and setae except for a mass of hooks or cincinnuli along the distal lateral margin. The cincinnular mass gives the appearance of a fingernail, while the appendix interna resembles a finger. The appendix masculina of a mature male is as large as or one and a half times as large as the appendix interna. Its distal end and ventral surface are covered with broad spines. The function of these two rami in the transfer of the spermatophores is not clearly understood. During the adult development of the male phase of *Pandalus platyceros,* both of these pairs of copulatory organs undergo striking morphological changes which can be subdivided into the following stages of development (Fig. 1).

Stage 1. This stage is typical of those individuals from September through April below 2.0 cm in carapace length. The copulatory organs of the first pleopods have not yet formed (Fig. A), although the distomedial surface is free of setae and indicates the location of the future organ. The appendices internae of the second pleopods are already well formed at this stage. Their distal ends display numerous cincinnuli. However, the appendix masculinae consists of a small bud or blastema with two minute spines at the distal end. (Fig. A').

Stage 2. Individuals between 2.0 and 2.5 cm carapace length between October and April comprise this stage. The copulatory organs of the first pleopods are

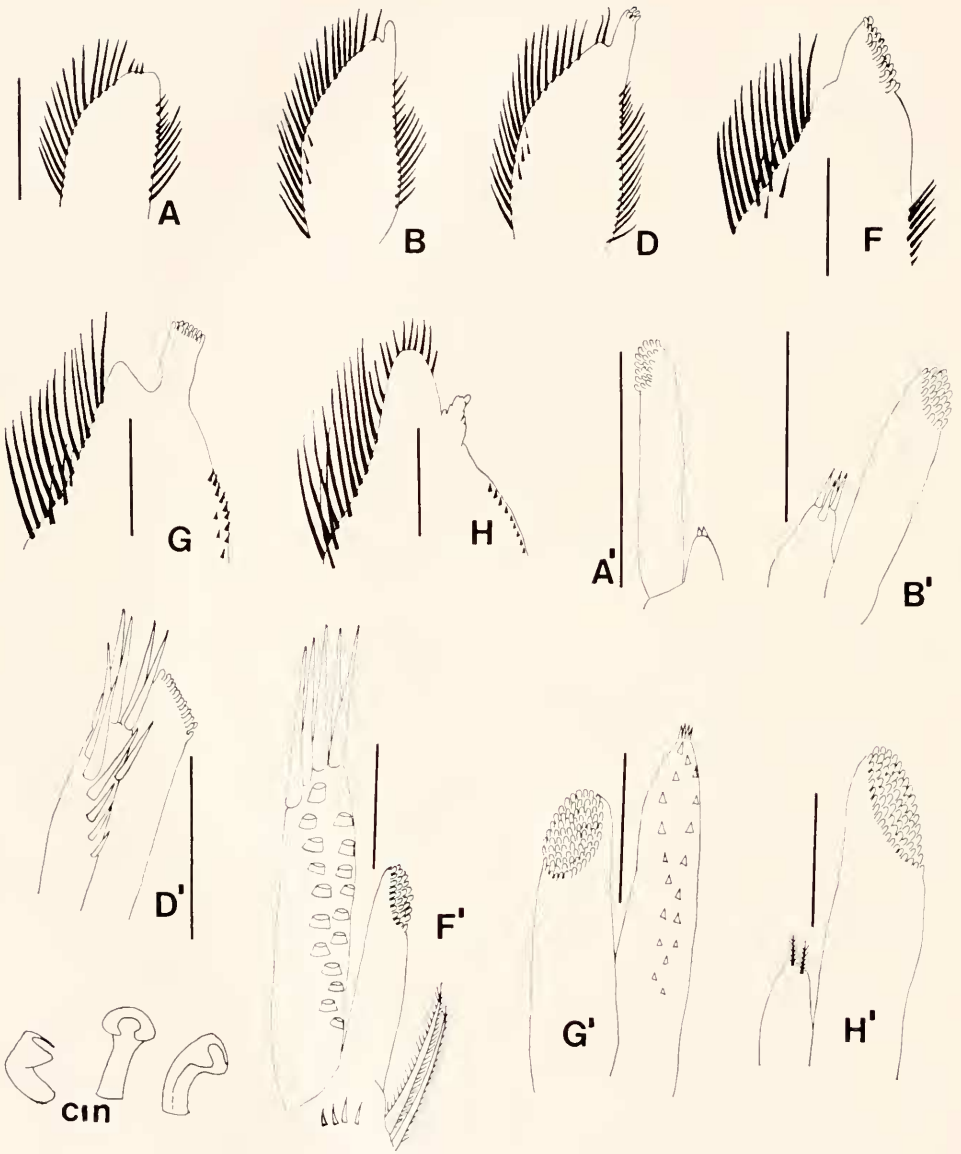

FIGURE 1. Copulatory appendages of male and transforming *Pandalus platyceros* Brandt; A to H, copulatory organs of endopodites of the first pleopods; A′ to H′, appendices internae and appendices masculinae of the endopodites of the second pleopods. Lateral spines of F′ have been cut to show their relative position. The scale is given by one millimeter slash marks. The cincinnuli, *cin,* are approximately 50 μ in length.

represented by a small flap or lobe on the distomedial edge of the endopodites. The lobe is free of cincinnuli (Fig. B). The appendices masculinae of the second pleopods (Fig. B′) are now almost half the length of the appendices internae.

The four apical spines that are typical of all appendices masculinae of larger forms are now present; however, they are much smaller (200 microns) than the spines of larger forms.

Stage 3. This stage is intermediate between 2 and 4 and perhaps may be skipped entirely in the developmental process since only a relatively small number is seen during the year. Individuals measuring 2.0 to 2.2 cm in May and June appear to be in Stage 3. The copulatory organs of the first pleopods are similar to those of Stage 2, however the appendices masculinae of the second pleopods are more than half the langth of the appendices internae. There is also a row of short spines that proceed obliquely down the ventral surface of the ramus, in addition to the four distal spines.

Stage 4. This stage represents individuals that range in size from 2.5 to 2.9 cm from November to April; and from 2.3 to 2.8 cm from May to September. This stage is typical of the summer non-reproductive males. The copulatory organs of the first pleopods now have a small number of cincinnuli at the apical end (Fig. D). The appendices masculinae are almost as large as the appendices internae (Fig. D′). In addition to the four apical spines which are now 500–600 microns in length, there is a row of six spines that run obliquely down the medial edge of the ramus. One or two spines may also be present on the ventral surface.

Stage 5. During the months of May through August this stage is found in the individuals measuring 2.9 through 3.5 cm that are producing sperm and functioning as males. For the most part those in this size range remain at this stage until late February and April when they attain Stage 6. Most of these individuals will function as males the following summer. Some, however, transform into females and into Stage 8 during March and April. The copulatory organs of the first pleopods are well developed and each has the appearance of a thumb-like projection from the disto-medial surface of the endopodite. There are many cincinnuli present at the apical end. The appendices masculinae are as large as or slightly larger than the appendices internae. There are from fifteen to eighteen broad spines present at the apical end and along the ventral surface. The largest of these spines has a length of from 700 to 800 microns. During January, the appendices masculinae of individuals of Stage 5 have shorter spines, measuring 300–400 microns. Probably a molt has occurred during November or December.

Stage 6. This stage represents the summer males measuring 3.5 to 4.2 cm that are functioning as males for the third summer. This stage first appears during February and March in the 3.1 through 3.8 cm size class males. The peak of development is reached from August to November. By January it appears that all of these individuals have molted and reached Stage 8. The copulatory organs of the first pleopods (Fig. F) are broad and triangular and each contains many cincinnuli along the medial margin. The appendix masculina (Fig. F′ of the second pleopods is at least $1\frac{1}{2}$ times as long as the appendix interna and contains 18 to 23 spines at the apex and along the ventral surface.

Stage 7. After undergoing molts during the months of December and January, the individuals measuring 3.4 to 4.3 cm are in the process of transforming from males into females. The copulatory organs of the first pleopods are now beginning to regress (Fig. G). The apex is free of setae and the organ itself is once more stalked and contains less cincinnuli than the previous stage. The medial end of

the endopodite is free of large setae, only very small stub-like setae remain. The appendix masculina (Fig. G') contains very reduced spines and its length is nearly equal to the appendix interna.

Stages 8 and 9. These two stages are actually variations of the same stage. The copulatory organs are typical of those individuals above 3.5 cm carapace length that have transformed into females. This stage first appears after the May or spring molt. The animals are now fully transformed individuals. The copulatory organ of the first pleopod (Fig. H) has atrophied to a widened stump on the inner surface of the endopodite. The distal end of the endopodite has become pointed and displays small setae along its margin. The small spines that were present in the male along the inner margin have disappeared. The appendix masculinae also has begun to atrophy. It may be present as a ramus that is almost the length of the appendix interna or it may be reduced to a small papilla which displays one or two small plumost setae (Fig. H'). In *Pandalus platyceros* this rudiment of the appendix masculina may be present in the ovigerous female. Although the appendix interna does not increase in length in the female stage, it becomes more robust, increasing in width by almost one millimeter. The cincinnuli of the appendix interna of the female do not disappear; they, in fact, appear to increase in number. In addition to these changes, the pleura of the abdominal segments lengthen at this stage to cover the pleopods and eventually the attached eggs.

The gross morphology of the gonad and accessory ducts

The gonad of *Pandalus platyceros* in the dorsal thoracic hemocoel consists of a pair of tube-like arms that are joined by a transverse bridge of tissue one-third the distance from its anterior end. In addition, the posterior ends of the arms are connected to each other by sheaths of squamous cells that encapsulate each arm of the gonad. Three-fifths from the anterior or distal end, along the lateral surfaces, a pair of sperm ducts take their origin. In immature males the ducts are relatively uncoiled and each terminates at the male gonopore at the base of the coxa of the eighth thoracic appendage. In mature males, the sperm ducts can be further differentiated into three regions. Proximal to the gonad is the tightly coiled efferent tubule which comprises approximately 25% of the total length. Next there is a muscle-sheathed sperm duct proper which gradually increases in diameter as it approaches the distal end, the highly muscular ejaculatory bulb.

During the male stages, the ovotestis has a central medulla of ovarian tissue and a cortex of testicular tissue. Only during the female stages does the gonad become a true ovary lacking cortical testicular tissue.

A pair of thin-walled oviducts originate about half way from the anterior end of the gonad, just anterior to the origins of the sperm ducts. Each proceeds ventrally from the origin to insert on the gonopore which is located on the base of the coxa of the sixth thoracic appendage. The oviducts are present during the male stage although they are inconspicuous and difficult to distinguish from blood vessels.

The ovotestis extends anteriorly to the first or second thoracic segment near the dorsal projection of the cardiac stomach and posteriorly beneath the pericardial

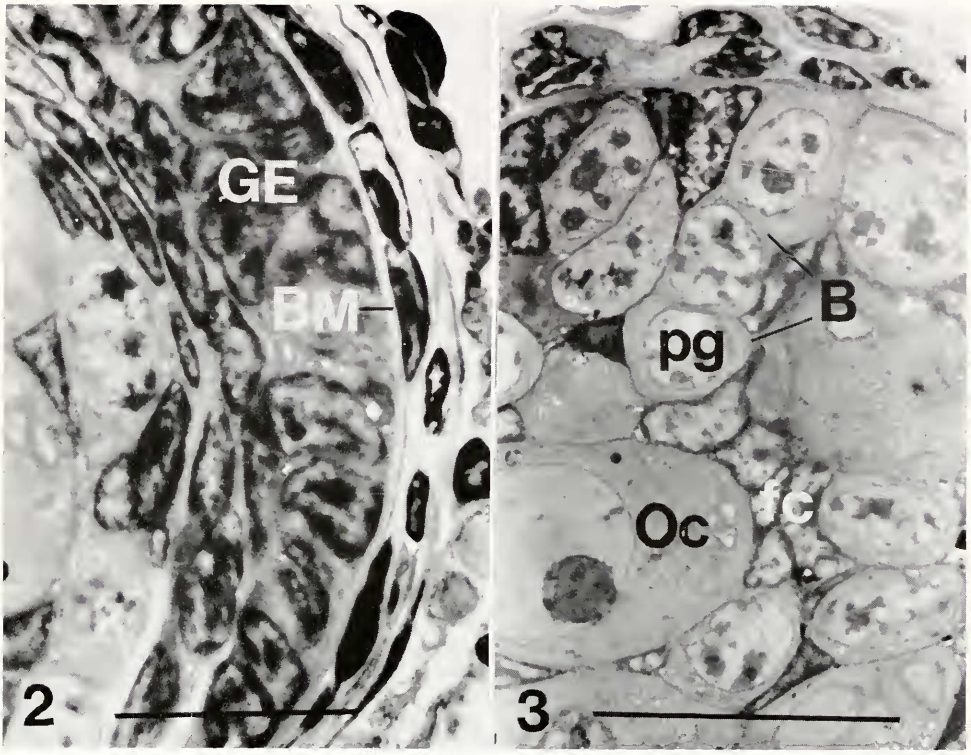

FIGURE 2. Transverse section (2 μ) through the gonad of a 2.3 cm CL male (October) showing gonodal epithelium (GE) and its basement membrane (BM). The slash mark represents 20 microns; glutaraldehyde-osmium fixation, Richardson's stain.

FIGURE 3. Transverse section (1 μ) through the ovarian germinal ridge of a 2.4 cm CL male (December). glutaraldehyde-osmium fixation, Richardson's stain. The slash mark represents 30 microns; B, intercellular bridges, fc, follicle cells, Oc, primary oocyte, pg, primary oogonial cells.

septum to the eight thoracic segment. A large hepatopancreas supports the gonad ventrally. During the latter stages of vitellogenesis, the ovary nearly fills the dorsal thoracic hemocoel, depressing the hepatopancreas and extending anteriorly into the subrostral hemocoel and posteriorly into the first abdominal segment.

The dorsal and lateral surfaces of the anterior half of the male stage gonad contains many red pigment cells or erythrophores in the capsular sheath. Whereas the posterior dorsal surface that lies beneath the pericardium usually remains unpigmented. During the female stage, in addition to a large number of erythrophores, there are numerous white pigment cells or guanophores in the sheath imparting a milky pink color to the gonad. Although the cells of the male stage gonad generally remain translucent and colorless, the olive green color of the eggs can be seen through the pigment cell layer of the ripe ovary.

Both ovotestis and ovary are anchored to the posterior lateral walls of the thoracic pleura by a pair of mesenteric sheaths that underlie the pericardium and overlie the posterior surface of the hepatopancreas. The anterior end of the gonad

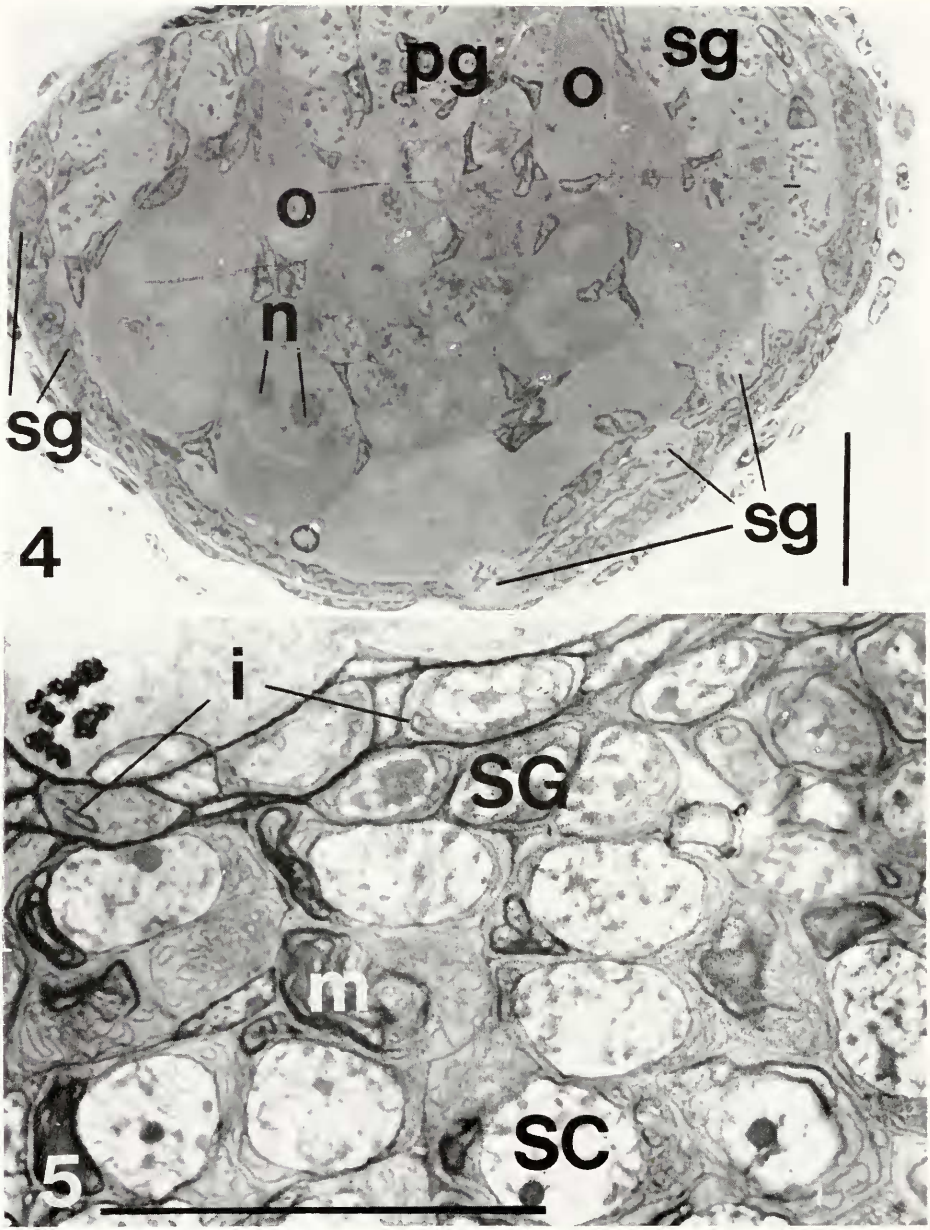

FIGURE 4. Transverse section (1 μ) through the ovotestis of a 2.4 cm CL male (March). Note that the primary oogonial cells (pg) are separated from the spermatogonial cells (sg) by the oocytes (o) that remain attached to the basement membrane of the gonodal epithelium by stalks; glutaraldehyde-osmium fixation, Richardson's stain. The slash mark represents 50 microns, n, nucleoli of oocytes.

FIGURE 5. Transverse section (1 μ) through a region of the cortex of the ovotestis of *Pandalus platyceros*. Note the well developed cytoplasmic inclusions (m) in the young

is attached to the anterior rostral region of the cephalon by strands of connective tissue that underlie masses of lymphogenous tissue. In addition, the saddle shape heart adds further support by sending a number of blood vessels over and into the gonad.

Development of the ovotestis and formation of the germinal ridge

Each gonadal arm has a cortical layer of epithelial cells from which all other gonadal cells originate. In five to eight month old individuals this layer may be several cell layers thick and containing large, irregularly shaped and densely staining nuclei (Fig. 2). Because of the paucity of cytoplasm in this layer and the apparent absence of cell boundaries, the nuclei of this germinal epithelial layer appear to reside in a syncytium.

Peripheral to the germinal epithelial layer, there may be several layers of highly squamous cells. These cells appear to be an epithelium which also contains the numerous pigment cells. They are separated from the gonad proper by the basement layer of the germinal epithelium (Fig. 2). It appears that this cellular layer may be formed extrinsic to the gonad, at least in the initial stages of male gonad development.

By the time a shrimp reaches a carapace length of 1.7 cm each arm of the gonad is composed of a cortex of cells that is derived entirely from the gonadal epithelium and a medula of primary gonial cells. These primary gonial cells differentiate into the follicle cells and oocytes to form the ovarian portion of the gonad. The medial gonadal epithelium or the germarium proper gives rise to these medullary cells. The medulla is characterized by at least two cell types. The first cell type is similar in morphology to the cells of the gonadal epithelium. Cells of this type are the ovarian follicle cells. They are generally irregular in outline and consist mainly of nucleus with dense chromatin granules (Fig. 3). These cells appear to originate both from the germarium proper and also from the regions of the gonadal epithelium that surround the medulla. They do not appear to undergo any mitotic activity but simply move off the basement membrane and into the ovarian medulla where they become associated with the oocytes. The second morphological cell type is the primary gonial cell which also appears to be formed from the dense nuclear cells of the germinal epithelium (Fig. 3). Mitotic figures are rare in these cells. Increases in both nuclear and cytoplasmic volume occur after these cells move off the basement membrane of the epithelium. At this stage the cells have an average diameter of 15 microns. Many of these cells appear to have intercellular bridges. Their nuclei have a tendency to become highly lobated with large numbers of nucleoli and densely clumped chromatin. A few of the primary gonial cells do not lose contact with the basement membrane and become flask-shaped. These cells move laterally as more gonial cells are proliferated from the germinal ridge or germarium. However they still retain their connection with the basement membrane by a slender neck of cytoplasm (Fig. 4). With the further increase in cytoplasmic volume the nuclei have many large nucleoli. At this stage of

spermatocytes (SC). Also note the early development of these inclusions (i) in the spermatogonial cells (SG); glutaraldehyde-osmium fixation, Richardson's stain. The slash mark represents 50 microns.

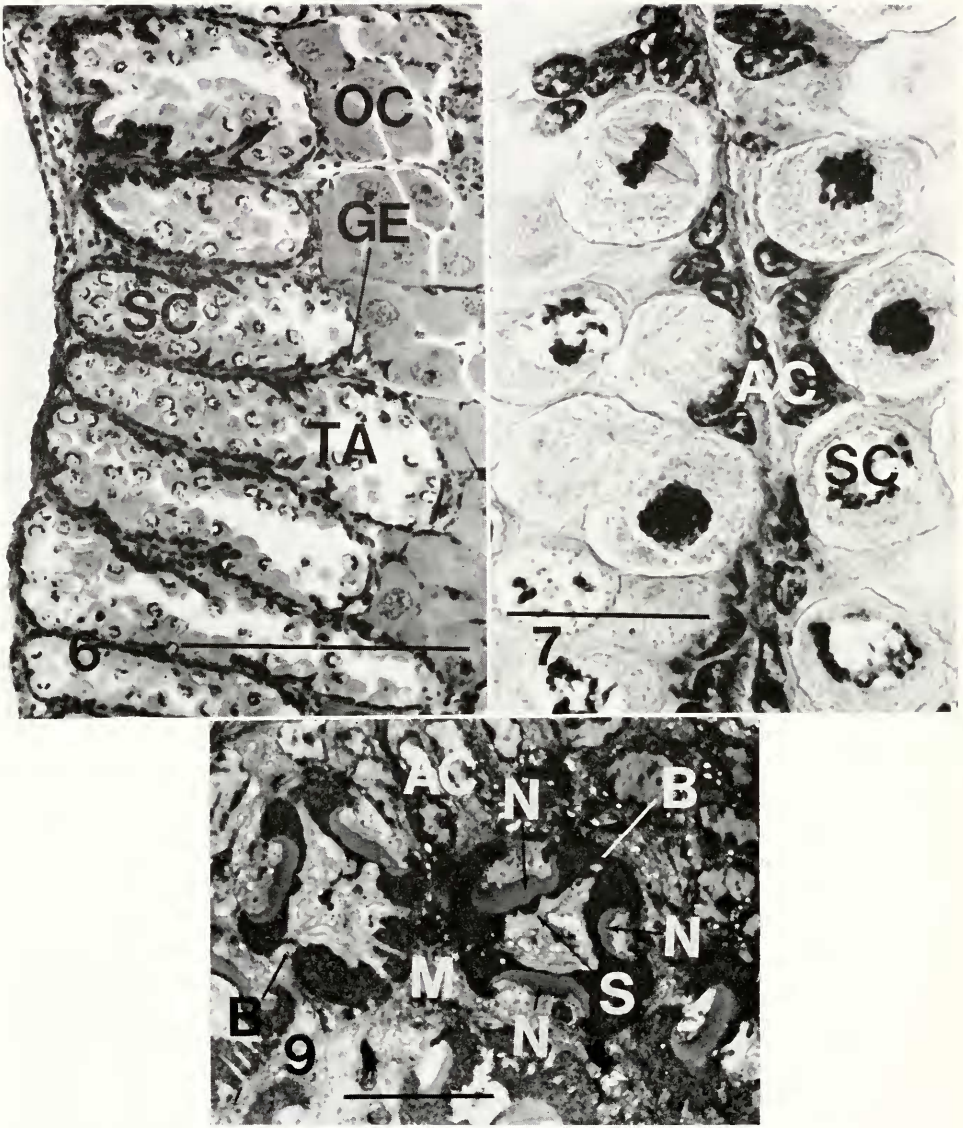

FIGURE 6. Frontal section (6 μ) through the ovotestis of a 3.6 cm CL male (June). Note the subdivision of the testicular cortex into a number of acini (TA) by the evagination of the gonodal epithelium (GE). The inner portion of this epithelium forms the lateral germinal ridge which gives rise to the spermatogonia; Hematoxylin–Halmi stain. The slash mark represents 500 microns, OC, oocytes, SC, spermatocytes.

FIGURE 7. Frontal section (6 μ) of a region of the testicular cortex of the gonad of a 3.1 cm CL male (June). Note the acinar cells (AC) projecting into the acinar lumina which are filled with spermatocytes (SC) undergoing synchronous maturation activity. Azan stain. The slash mark represents 50 microns.

FIGURE 9. Frontal section (1 μ) through two tetrads of spermatids in a testicular acinus of *Pandalus platyceros*. Note the three spermatids joined together by intercellular bridges (B).

development, the cells become primary oocytes and may measure 30–40 microns in diameter. As the cytoplasmic volume of the oocytes increases, the cell moves deeper into the ovarian medulla although still attached by the stalk to the basement membrane (Fig. 4). Finally, the stalk appears to break and the oocyte takes up a position in the peripheral region of the medulla. The primary gonial cells that appear to dissociate from the germinal ridge comprise the central region of the ovarian medulla. These cells possess nuclei in various stages of mitotic prophase and thus have yet to differentiate into primary oocytes. In addition, there are numerous cells with lobate nuclei which appear to be in association by intercellular cytoplasmic bridges with each other and with primary oocytes (Fig. 3). These cells may be nutrient or nurse cells. Whether or not these cells differentiate into oocytes could not be determined through histological sections.

In those individuals that attain a carapace length greater than 2.2 cm by November of their first year, there is cellular differentiation in the gonadal epithelium lateral to the ovarian germinal ridge. As in the case of the oogonial cells, the cytoplasmic volume of these lateral cells begins to increase and the nuclei take on a lobate shape. These cells do not remain attached to the basement membrane but move into the tube or arm of the gonad lateral to the ovarian medulla. The cytoplasm of these cells contains dense staining inclusions that have the appearance of microtubules in glutaraldehyde-osmium fixed tissue (Fig. 5). These cells appear to be the spermatogonia. Once they have taken their position within the cortical region of the gonad, most of these cells undergo asynchronous mitotic division into secondary spermatogonial cells. By this stage the nuclei of the cells are in various stages of mitotic activity. Many of these cells will divide again, and with each division will move more laterally.

After the mitotic divisions are completed, the cells have increased in size to 40 microns. The swirls of microtubules have increased to fill the cytoplasm (Fig. 5). These cells have now become primary spermatocytes since no mitotic activity is evident. Many of these primary spermatocytes appear to be connected to each other, perhaps by remnants of spindle fibers. The chromosomes appear to be in a pre-leptotent stage. No nucleoli are evident. The nucleus reaches a maximum diameter of 40 microns; the cell itself reaches a diameter of 50 to 60 microns. When primary spermatocytes are formed by first year individuals during the winter and early spring, these germ cells degenerate and a new crop of primary spermatocytes take their place. Individuals of less than 2.2 cm carapace length in November appear not to produce primary spermatocytes until late summer (August–September).

With the formation of spermatogonia from the lateral germinal ridges, there is a lifting or elevation of the lateral edges of the gonodal tube. The dorso-medial surface that comprises the ovarian germinal ridge appears to be held down by the flasked shaped oocytes that have the major portion of their cell masses anchored within the medulla of the gonad. The ovarian germinal ridge also begins to protrude outward due to the mechanical pressure of the uplifting of the lateral edges,

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The fourth spermatid of each tetrad is out of the plane of section. Note also that the spermatid tetrads are surrounded by mucous (M) secreted by the acinar cells (AC); glutaraldehyde-osmium fixation, Richardson's stain. The slash mark represents 25 microns, N, nucleus of spermatid, S, developing spine of spermatozoon.

TABLE I

The relationship between age and carapace length to state of gonadal development in Pandalus platyceros *Brandt*

Age in months	Sex	Carapace length (cm)	State of gonadal development
0– 5 (Apr.–Aug.)	—	Planktonic larvae	----------
5– 7 (Sep.–Nov.)	Imm.	1.2–2.2	Oogonia and oocytes; no spermatogonia
7–14 (Nov.–Jun.)	♂	1.7–2.2	Oogonia and oocytes; few spermatogonia after Jan.
7–16 (Nov.–Aug.)	♂	2.2–2.8	Spermatogonia and spermatocytes present; average oocyte 60 μ
13–16 (May–Aug.)	♂	2.9–3.5	Spermatids present; spermiogenesis; average oocyte 100 μ
16–19 (Aug.–Nov.)	♂	2.7–3.3	Spermatogonia and spermatocytes; no spermatids; average oocyte 120 μ
16–19 (Aug.–Nov.)	♂	3.3–3.7	Testicular tissue degenerates; increase in oogonia and oocytes, average oocyte 250 μ
19–24 (Nov.–Apr.)	♂	3.0–3.8	Increase in proliferation of spermatocytes; average oocyte 130 μ
19–24 (Nov.–Apr.)	Trans.	3.5–4.0	Increase in proliferation of oocytes and primary oocyte growth; average oocyte 370 μ
24–28 (Apr.–Aug.)	♂	3.5–4.0	Spermatids present; spermiogenesis; average oocyte 180 μ
24–28 (Apr.–Aug.)	♀	4.2–4.5	Increase in oocyte growth with deposition of proteinaceous and lipid yolk platelets; average oocyte 900 μ
28–31 (Aug.–Nov.)	♀	3.7–4.2	Testicular tissue degenerates; increase in oogonia and oocytes; average oocyte 250 μ
28–31 (Aug.–Nov.)	♀	4.5–4.9	Spent ovary; ovigerous females

now called lateral wings of the gonad. The lateral wings are formed because of the increased production of spermatogonia and spermatocytes which move out laterally as more spermatogonia are produced from the lateral germinal ridges. In addition, there are formed a series of lateral evaginations of the dorso-lateral gonadal epithelium which compartmentalizes the gonodal tube into a series of cortical testicular acini (Fig. 6). These evaginations greatly increase the surface area of the lateral germinal ridges which result in an increased production of spermatogonia. In addition, the infoldings and evaginations of the lateral germinal epithelium causes the ovarian and testicular germinal ridges to take up a central position within the gonadal tube, completely surrounded by the testicular acini. Although the lateral germinal ridges of the gonadal epithelium form most of the spermatogonial cells, some spermatogonial cells may be found along the basal part of the gonadal epithelium.

There are two age classes of males present during the winter and spring months (Table I). The first age class that hatched the previous year during April and May consists entirely of males. Most of these shrimp will function as males during the coming summer, only those below 2.2 cm in carapace length in January that have not yet produced any spermatogonia will not produce spermatozoa during their second summer. Their first summer is spent in the plankton as pelagic zoea larvae. The second age class is composed of shrimp that hatched during the spring two years previously. Most of the shrimp below 3.5 cm in January will function

as males during the coming summer. Those above 3.5 cm and a very few between 3.2 and 3.5 cm are in a transitional stage and will function as females in the coming summer.

During the winter months, growth nearly is arrested in both age classes of *P. platyceros*. Similar conditions have been reported for *Pandalus kessleri* (Aoto, 1952) and *Leander serratus* (Forster, 1951). The gonads of specimens of *P. platyceros* below 2.2 cm carapace length show little indication of spermatogonia formation. However, by late March or early April, when growth is once more underway, spermatogenesis is initiated. In these same animals oogenesis began in early autumn and is still progressing since the primary oocytes have grown to an average diameter of 50 microns. Usually these oocytes degenerate in these immature forms. In those individuals above 2.2 cm of the first age group, spermatogonia are evident in the cortex of the gonad as early as November of the previous year. Thus, these individuals are about seven months old when spermatogonia formation is initiated. By January, a 2.4 cm individual, the cortical region of the gonad is filled with spermatogonia and spermatocytes and the medullar oocytes have attained an average diameter of 60 microns. By the end of June, a 2.9 cm shrimp has large oocytes with an average diameter of 100 microns.

Although primary spermatocytes are abundant in the testicular acini of first year age group during the winter and spring months, the maturation divisions have yet to occur. The nuclei of the spermatocytes are arrested at the leptotene and zygotene stages of prophase. As new populations of spermatogonia and spermatocytes are produced, the oldest spermatocytes undergo degeneration and younger spermatocytes take their place in the acini. At this time the gonodal epithelium that surrounds the testicular acini, the acinar cells of the testis, is composed of highly squamous cells with flattened nuclei. These squamous cells are characteristic of the gonodal epithelium of the testis during the nonreproductive seasons.

Almost all individuals that have a carapace length less than 2.5 cm in June do not produce sperm during the summer months; however, they will become reproductive during the following summer. By September, these same individuals reach a maximum carapace length of 3.1 cm and their sperm ducts contain no spermatozoa.

Maturation of the spermatocytes and spermiogenesis

The actual time of sperm formation is directly related to the size of the shrimp. The larger the male the earlier sperm formation begins. Males entering their third spring initiate the maturation processes as early as April. Those second year males that produce spermatocytes the previous autumn and winter begin to produce secondary spermatocytes as early as May. Also, any second year males that have not produced spermatocytes until early spring generally do not demonstrate meiotic spermatocytes until late July or early August. By September the males with a carapace of 2.7 cm or less, do not produce sperm until their third summer when their carapace length ranges from 3.5 to 4.0 cm (Table I).

The first indication of testicular meiotic activity is the proliferation and hypertrophy of the acinar cells of the testis. The cortex of the testis becomes greatly distended with spermatocytes which in turn appear to form an association with the acinar cells which project into the acini (Fig. 7). Wave after wave of spermatogonia are produced. Finally, only primary spermatocytes fill the acini. No longer

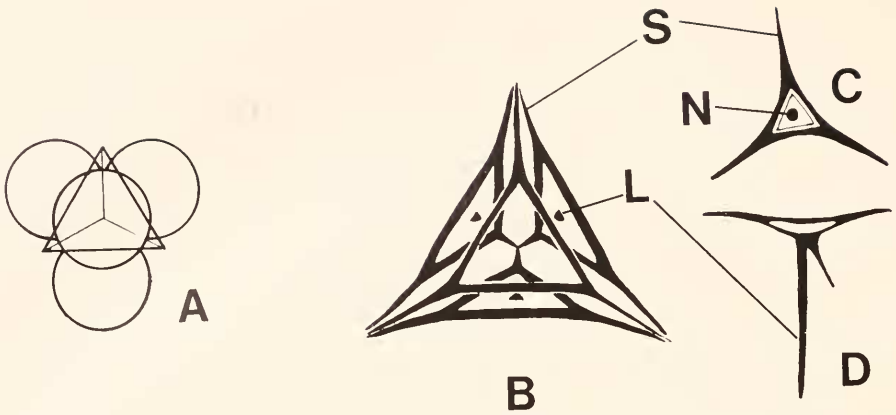

FIGURE 8. Spermiogenesis in *Pandalus platyceros* Brandt; (A.) the tetrahedral configuration of daughter spermatids from the same mother spermatocyte; (B.) diagrammatic representation of the origin of the triangular shaped spermatozoon based on the tetrahedral arrangement of daughter spermatids. The developing large spine, L, projects inward; s, short lateral spines; (C.) frontal view of a spermatozoon with the large spine projecting from the page; n, nucleus; (D.) side view of the spermatozoon. The scale is given by the large spine which is 40 microns in length.

are any spermatogonia produced by the germinal ridges. This cessation in the formation of spermatogonia coincides with the change in morphology of the acinar cells. The first meiotic division approximately is synchronous. The second meiotic division closely follows the first; in fact, actively dividing primary and secondary spermatocytes are evident in the same acinus. The spermatids that are derived from the same mother spermatocyte will remain in association with each other. During the latter stages of meiosis, nuclear condensation occurs with the extrusion of nucleoplasm into the surrounding medium. The four spermatids joined by intercellular bridges form a tetrahedron with each of the flattened nuclei forming the sides of the tetrahedron (Fig. 8). Fawcett, Ito and Slautterback (1959) demonstrated that cells such as spermatocytes that undergo synchronous division are connected to each other by such intercellular bridges. At this stage the spermatid tetrads are deeply surrounded by the acinar cell mucous (Fig. 9). The spermatid begins to flatten out, each forming a triangular side of the tetrahedron. The cytoplasm secretes a chitinous sheath surrounding the highly flattened nucleus. The tips of each triangular shaped cell project outward to form small spines. A large chitinous spine ultimately measuring 40–50 microns develops from the inner side of the spermatid. As each of the large spines of the four maturing spermatozoa grow inward they probably disrupt the tetrahedral arrangement. At this stage the acinar cells begin to regress to form a cuboidal epithelium lining each acinus. The lumen of each acinus is then filled with spermatozoa and mucous which is highly PAS positive and also paraldehydefuchsin positive. Finally, the acini evacuate their contents into the sperm ducts which are continguous with the testicular acini. It appears that the acinar cells are similar in function to the mammalian prostatic cells in that their product functions in the evacuation and perhaps maintenance of the immotile spermatozoa from the testis into the sperm ducts.

The transformation of the male gonad

Once spermiogenesis is completed and the sperm ducts are filled with spermatozoa, there is a tendency for the males to initiate the transformation into the female phase. The testicular gonadal epithelium (acinar cells) has lost its ability to form more spermatogonia since it has differentiated into mucous producing cells during spermatogenesis. Post-spermatic males may initiate gonadal transformation either when they are 16 to 19 months old, or, if they did not produce spermatozoa during their second summer, when they are 28 to 31 months old (Table I). As early as late July the testicular epithelial cells begin to degenerate and form scar-like connective tissue. The lumina of the acini are obliterated by newly formed and growing primary oocytes which push the degenerating testicular tissue against the squamous tissue sheath of the gonad. This atrophying testicular tissue forms the thick outer sheath of the female gonad. At the same time, many white chromatophores make their appearance in this outer sheath along with the numerous red chromatophores that were present during the male stage. Most of the large oocytes (100–200 microns) that were formed during the male stage are also in a state of degeneration and appear to be phagocytized by their surrounding follicle cells. Baffoni (1960) reported that the follicle cells of female decapod gonads do phagocytize oocytes following ovulation. The areas left by the degenerating oocytes are soon filled by new primary oocytes proliferating from the median germinal ridge.

After the sperm ducts have filled with spermatozoa and mucous, the epithelial layer of the ducts become tall and columnar. Paraldehydefuchsin granules appear in the cytoplasm and ultimately the cells secrete a cuticular sheath around the sperm mass and then regress to form a highly squamous epithelium. During copulation, the outer circular muscle of the ducts contract forcing a cuticular enveloped strand of sperm through the gonopores.

By November, all of the transforming shrimp have the external sex characteristics of functional males (stages 5 and 6). These male characteristics are maintained during the winter months relatively unchanged, but the gonads during this time are totally ovarian. Thus the shrimp have the primary sex organs of true females but the external secondary sex traits of males.

Discussion

The gonads of the higher crustaceans are similar to those of mollusks in that both are covered by a germinal epithelium (Raven, 1961). There is some confusion in the early literature as to the role of this epithelium in the production of gonial cells. Grobben (1878) noted an epithelial coat on the gonads of all the male decapods that he studied. He believed that certain regions of this epithelium gave rise to the male germ cells. The remainder of the epithelium has the function of secreting the spermatic plasma that envelops the mature sperm. Because the germinal region which he called the "Ersatzkeim" contained so many densely packed nuclei of irregular outline and form he described it as a syncytium. He even believed that many of these nuclei were undergoing amitoses. This statement was based on nuclei which were dumbbell shaped and thus appeared to be splitting in two. However, since the nucleus itself is not a rigid structure but rather a simple structure surrounded by a double membrane, the tight packing of these nuclei

into a tubular structure would cause them to become highly distorted. Such distorted nuclei are present in the gonadal epithelium of *P. platyceros* especially during the juvenile male stages when the nuclei are stratified.

Gilson (1884) also stated that the "metrocytes" or sperm mother cells appear to be formed from the "Ersatzkeim" or replacement blastema. He also described this region as a synctium, however, he used the term plasmodium which is really not applicable to animal tissue. From both wax and thick epon sections it is difficult to disprove that the replacement blastema is a synctium. But this layer does possess a basement membrane which is typical of an epithelium. Because of the paucity of cytoplasm surrounding the nuclei, the gonadal epithelium and replacement blastema may appear to be a syncytium.

Another observation of both Grobben (1878) and Gilson (1884) was the lack of mitotic figures in the gonadal epithelium and especially in the region giving rise to the spermatogonia. I also have not found a preponderance of mitotic figures present in the epithelial layer and especially the germinal ridge (replacement blastema). However, mitotic figures are infrequently found in the germinal epithelium of *P. platyceros*. The paucity of such figures may indicate a rapid rate in the mitotic cycle. This epithelial layer is reminiscent of the androgenic gland tissue which also has a proclivity toward unseen mitotic activity. Sabatier (1885) reported that the spermatocytes which he called protospermatoblasts are derived from the connective tissue layer that forms the skeleton of the tubule in decapods. The epithelial layer of the gonad of *P. platyceros* is separated from the squamous tissue sheath of the gonad by a basement membrane. It, therefore, is improbable that such a layer of cells could produce spermatogonia into the lumen of the gonad. Initially, the squamous tissue sheath of the gonad appears to be formed extrinsic to the gonad perhaps by wandering scleroblasts.

The gonads of natantian decapods resemble those of amphipods more than other decapods. Meusy (1963) also noted in *Orchestia gammarella* that the zone of proliferation of spermatogonia is limited to a certain region of the gonadal epithelium. This germinal ridge appears to be a syncytium according to Meusy.

In protandric mollusks such as *Crepidula*, the entire germinal epithelium alternatively first gives rise to male and then female sex cells (Coe, 1936). But in a protandric hermaphrodite, such as *Pandalus* in which both male and female gametes can be produced simultaneously, there are questions remaining on the mechanism of the production of these two cell types. Aoto (1952) working on *P. kessleri* stated that the germinal ridge that runs down the medio-lateral surface gives rise to oogonia whereas the spermatogonia have a different origin within the interstitial cell masses that encapsulate the central ovarian mass. These interstitial cell masses are similar to the lateral germinal ridges of *P. platyceros* following the uplifting of the testicular acini. Berreur-Bonnenfant and Charniaux-Cotton (1965) observed in *P. borealis* that there is one germinal ridge per gonadal tube which alternatively gives rise to spermatogonia and oogonia. They explain this alternation in germ cell production as being due to a difference in androgenic hormone titer. When the titer is high, spermatogenesis occurs; when it is low, oogenesis. The observations on *P. platyceros* do not corroborate these observations. There are two distinct germinal areas that run down the gonadal tube. Each ridge is separated at all times from the other by large primary oocytes that remain attached to

the basement membrane until others generally take their place. Although such stalked oocytes are evident in the drawings of Berreur-Bonnenfant and Charniaux-Cotton (1965), they do not attach any significance to them. Such histological data present in their paper is used to define a physiological manifestation; i.e., change in hormone titer which this author feels is presently undefinable due to lack of a reliable bioassay (Hoffman, 1969). In addition, no data is given as to the age or season of the year of the shrimp used in their analyses. Alternation in oogenesis and spermatogenesis from the medial germinal ridge has never been observed in male stage of *P. platyceros* under natural conditions. Both phenomena appear to occur simultaneously. During the adult male stages there is an increase in the number of spermatogonia that are proliferating from the lateral germinal ridges, but oogenesis is still occurring, albeit not at the same rate as spermatogenesis. Oogenesis is a continuous process in *P. platyceros* since young oogonia are always present in the ovarian medulla of male individuals. This observation is similar to the observations made on the ovotestes of hermaphroditic mollusks such as *Helix* (Ancel, 1903; Gatenby, 1917).

Of important note is the large size of the primary spermatocytes of *P. platyceros*. It is relatively rare that animal spermatocytes ever attain a diameter of 40 to 60 microns. I believe that these cells actually may be oocytes modified through the action of a male hormone from the androgenic glands. If we assume that protandry in pandalids arose from gonochoristic stock, natural selection would tend to favor the female stages as the originator of the protandric evolutionary line. The male stage could be considered as the "juvenile" stage when little energy would be necessary for the formation of sperm. In addition, male stages could be smaller than females since they require little volume to produce and store sperm. The female then would be the "adult" stage. A large organism is required to produce a large number of large yolky eggs. Also, a large animal has a selective advantage especially when it must carry its fertilized eggs on its abdominal appendages over a period of several months. Berreur-Bonnenfant (1963) demonstrated that testicular cells of certain Malacostraca when cultured *in vitro* in the absence of androgenic glands will autodifferentiate into oocytes. Also, Charniaux-Cotton (1957) observed the anlage of androgenic glands in female talitrid amphipods. However, these anlage never become functional in the female. Therefore, female malacostracans do have the potential to differentiate into males. Through a series of exciting experiments Charniaux-Cotton (1959) has been able to reverse the sex of female talitrid amphipods by the implantation of androgenic glands. These experimentally reversed females not only produce viable sperm but will even copulate with other females. It could be argued that protandry arose from the male line instead of the female, but then such males would have had to develop a very complex series of mechanisms for the development and maturation of oocytes. This is something that is already innate in the female when the ability of some malacostracan oocytes to autodifferentiate is taken into account (Berreur-Bonnenfant, 1963).

Legrand (1964) has proposed the concept of gonad territory to explain how a single gonad might give rise to both male and female sex cells. In oniscoid isopods, Legrand observed that the axial part of the gonad gives rise to oogonia, while the utricular part forms the spermatogonia. The androgenic hormone

only then would have the function of initiating the development of the testicular utricles into which the germ cells migrate after they are first formed in the axial tube. A territoriality such as this may be set up in the germinal ridge of *Pandalus* subdividing it into a medial ovarian region and lateral testicular regions by the primary oocytes that have been formed in the early stages of gonad development before there had been any production of spermatogonia. Shrimp in their first autumn (five to eight months old) measuring 1.2 to 2.0 cm in carapace length have oogenesis well underway, although the oocytes only reach a maximum diameter of 40 microns. Spermatogenesis has yet to occur. These immature forms could be considered more female than male. At this same stage of development the androgenic glands are in their initial stages of development (Hoffman, 1969). Since the proliferation of oogonial cells in the gonad appears to precede androgenic gland development, the medial ovarian germinal ridge may not be susceptible to androgenic hormone when it becomes present. This territoriality could have been introduced when the shrimp are from five to eight months old. If the androgenic glands do not develop, the gonads could produce oocytes and this way result in the formation of primary females that are evident in certain pandalid species (Butler, 1964).

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Summary

1. The relationship between age and carapace length to the state of gonadal development in *Pandalus platyceros* Brandt is presented. The development of the ovotestis is shown to be a function of size (carapace length) and not age.

2. The copulatory appendages of the pleopods appear to pass through seven or eight stages from the immature form to the adult female stage.

3. The development of the ovotestis is described with special reference to the origin of gonial cells. It appears that oocytes and spermatocytes derived from two distinct germinal areas of the gonadal epithelium. The lateral germinal ridges give rise to spermatogonia and acinar cells; the medial germinal ridge, oogonia and follicle cells.

4. The flattened triangular spermatozoa are derived from the tetrahedrally arranged tetrads of spermatids.

5. Transformation to the female stage generally is initiated only after the males have produced spermatozoa. The lateral germinal ridges appear to have lost their ability to form spermatogonia since they have differentiated into prostatic-like mucous producing cells.

6. In the transforming stage, the degenerating cortical testicular tissue forms the thickened outer sheath of the ovary. Four months after spermatozoa forma-

tion, the sperm ducts are filled with sperm, the copulatory organs are still masculine; but the gonad lacks testicular tissue and becomes a true ovary.

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